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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
09/786,317	04/27/2001	Masatoshi Hagiwara	04276.00002	8076

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EXAMINER	
WALICKA, MALGORZATA A	
ART UNIT	**PAPER NUMBER**
1652	

DATE MAILED: 02/09/2004

Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary	Application No.	Applicant(s)	
	09/786,317	HAGIWARA ET AL.	
	Examiner	Art Unit	
	Malgorzata A. Walicka	1652	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If the period for reply specified above is less than thirty (30) days, a reply within the statutory minimum of thirty (30) days will be considered timely.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133).
- Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *08 September 2003*.

2a)☒ This action is **FINAL**. 2b)☐ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *4-13* is/are pending in the application.

4a) Of the above claim(s) *6-8 and 10-12* is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *4,5,9 and 13* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☒ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. §§ 119 and 120

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

13)☐ Acknowledgment is made of a claim for domestic priority under 35 U.S.C. § 119(e) (to a provisional application) since a specific reference was included in the first sentence of the specification or in an Application Data Sheet. 37 CFR 1.78.

a) ☐ The translation of the foreign language provisional application has been received.

14)☐ Acknowledgment is made of a claim for domestic priority under 35 U.S.C. §§ 120 and/or 121 since a specific reference was included in the first sentence of the specification or in an Application Data Sheet. 37 CFR 1.78.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO-1449) Paper No(s) _____ .
4) ☐ Interview Summary (PTO-413) Paper No(s). _____ .
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☐ Other: .

The Amendment and Response to Office Action, filed on September 08, 2003, is acknowledged. Claims 1-3 have been cancelled; new claim 13 has been added. Claims 4-13 are pending in the application. Claims 4-5, 9 and 13 are the subject of this Office Action. Claims 6-8, and 10-12 are withdrawn from consideration, 37 CFR 1.142(b), as being drawn to a non-elected invention.

Detailed Office Action

2. Objections

2.1. Specification

The objections to the specification has been withdrawn because a substitute specification in proper idiomatic English and in compliance with 37 CFR 1.52(a) and (b) has been filed. The substitute specification expands the abbreviations CREB, RSGFP, (RGFP) and BSGFP (BGFP).

The specifcation is objected to because improper description of Fig. 4-6 and the use of the term absorbance on page 25 (and in other pages). According to the description of axes, the figures present the intensity of fluorescence as a function of wavelength. It is not absorption of the monitor protein in dependence of wavelength.

2.2. Claims

The objection to claim 4 is withdrawn because the claim has been amended.

3. Rejections

3.1. 35 USC, section 112, second paragraph

Rejection of claims 1-3 made in the previous office Action is moot, because the claims have been cancelled.

Rejection of claims 4-5 and 9 is withdrawn because the claims have been mended and depend now on the new claim 13.

Claim 9 is rejected under 35 U.S.C. 112, second paragraph, as being incomplete for omitting essential step, such omission amounting to a gap between the steps. See MPEP § 2172.01. The omitted step is "and measuring fluorescence of the monitor protein before and after reaction with a test protein."

2.2. 35 USC, section 112, first paragraph

2.2.1. Lack of written description

Rejection of claim 1-3 made in the previous office Action is moot, because the claims have been cancelled.

Claim 4 –5, 9 and 13 are rejected under 35 U.S.C. 112, first paragraph, as containing subject matter, which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention.

Claims 13 and 9 are directed to a monitor protein, and its use, said protein comprising a) a phosphorylation region and b) a pair of fluorescent proteins, wherein a fluorescent protein of the pair is bound to each opposite end of the phosphorylation region. The claims are generic, because the terms "phosphorylation region" and "a pair of fluorescent proteins" are generic. Applicants provide the structure of two fused

proteins. These are two green fluorescent proteins from *Aequorea victoria,* the BSGFP protein and RSGFP protein, fused to the CREB fragment of SEQ ID NO: 1 (encoded in the plasmid pETIC-ART; ART stands for A-kinase Responsive Tracer) and the BSGFP protein and RSGFP protein fused to the phosphorylation sequence of SEQ ID NO: 2 of kemptide (Kempart, encoded by DNA construct comprised in the plasmid pETIC-Kempart); see page 18, the first two paragraphs. Of these two fused proteins only ART is a true representative species of the claimed genus of the monitor proteins, because of change in fluorescence resonance energy transfer when SEQ ID NO: 1 of ART is phosphorylated. In regards to the Kempart protein, Applicants disclose on page 7, line 6:

> "The fusion protein derived from "pETIC-Kempart" is phosphorylated, however, this phosphorylation does not generate protein conformational change necessary for generating changes in GFP fluorescence."

Thus Applicants' own data provide evidence that constructing the monitor protein according to claim 13 may not result in the claimed invention. Applicants failed to set forth any identifying characteristic of the other representatives of the genus.

In addition, as concerns claim 4, it is certain that not any fusion of RSGFP and BSGFP protein from *Aequorea victoria* to any phosphorylation region will produce a monitor protein as claimed in claim 4, **because the fusion protein obtained by expression of pETIC-Kempart is not a monitor protein**. The claim is directed to a genus of fusion proteins, however, the only fusion protein species disclosed by

Applicants consists of SEQ ID NO: 1 and the RSBFP and BSGFP, which is produced by expression of the plasmid pETIC-ART. The disclosure is silent as to what is an identifying characteristics of the phosphorylation region that is critical for changes in fluorescence of the fusion protein in result of phosphorylation of its phosphorylation region, when RSGFP and BSGFP are used as fluorescent protein.

Furthermore, with regards to claim 5, it is not certain that any fusion of any pair of fluorescent proteins to phosphorylation region of SEQ ID NO: 1 will produce a monitor protein as claimed in claim 5. The claim is directed to a genus of fusion proteins, however, the only fusion protein species disclosed by Applicants consists of SEQ ID NO: 1 and the RSBFP and BSGFP. The disclosure is silent as to what is an identifying characteristics of the pair of fluorescent proteins that is critical for changes in fluorescence of the fusion protein in result of phosphorylation region of SEQ ID NO: 1.

In conclusion, Applicants failed to describe the claimed invention in such full, clear, concise, and exact terms that a skilled artisan would recognize they were in possession of the claimed invention.

In response to the rejection for lack of written description Applicants, in their Remarks, write on page 10, line 2,

> "Other pairs of fluorescence proteins that can exhibit a change in fluorescence by interacting with each other are known in the art before the effective filing date of application, September 2, 1998. See exhibits A-B."

Applicants's arguments have been fully considered but are found not persuasive for the following reasons. Although the relevant art has been provided many examples of the fluorescent protein that theoretically might be used for construction of a fusion monitor protein consisting of a phosphorylation region flanked by equivalents of RSGFP and BSGFP proteins, Applicants' own data pinpoint that it is a particular combination of the sequence of phosphorylation region and the sequence of fluorescence proteins that may or may not act as a monitor protein. Fusing of RSGFP and BSGFP protein from *Aequorea victoria* to SEQ ID NO: 1 results in the monitor protein, however, when the proteins of the very same pair are bound to each opposite end of the phosphorylation region of SEQ ID NO: 2, the fused protein is not a monitor protein. Thus it is not predictable which combination of a phosphorylation region and fluorescence proteins will work as a monitor protein.

In addition the Applicants' opinion (page 11 line 22) is,

"The 'phosphorylation region' of the claimed monitor protein is also adequately described in the specification. The specification disclose relevant identifying characteristis of the phosphorylation region, i.e., its structural and chemical properties. The specification discloses that 'a "phosphorylation region" means a region comprising an amino acid residue to be phosphorylated and capable of changing its conformation by phosphorylation.' (Page 7, lines 21-23.)"

Applicants' argument has been fully considered but is found not persuasive. Firstly, the substitute specification does not contain the quoted passage on page 7, but on page 9, line 8. Secondly, on the same page, line 20, Applicants give the other definition of the phosphorylation region:

> "The phosphorylation region is preferably a partial sequence of a protein comprising an amino acid residue to be phosphorylated but can also be the full length protein. For instance, in the case of CREB transcription factor, it is known that the serine residue at amino acid 133 is phosphorylated by proteinase A. Therefore, any partial sequence of CREB transcription factor can be used as long as it contains the serine residue at amino acid 133 and is capable of being phosphorylated."

In this definition the Applicants do not require the conformational changes induced in the phosphorylation region by its phosphorylation. Furthermore it seems that the lenght of the phosphorylation region does play a role, as FRET is a short distance phenomenon.

Those skilled in the art realize that the fusion protein consisting of a protease cleavage region and a pair of fluorescent proteins, wherein a fluorescent protein of the pair is bound to each opposite end of the cleavage region, always works as monitor protein for the specific protease, because cleavage of peptide bond switches off the FRET between the fluorescent proteins. Similar system, however, does not always

work for a kinase because the conformational changes caused by phosphorylation are much more subtle than the cleavage of the peptide bond. Thus, they may or may not result in the change in the fluorescent properties of the fusion protein. Besides, the conformational changes in the phosphorylation region seem to be influenced by the amino acid sequence of the entire fusion protein.

In conclusion, the monitor protein of invention has to be identified by its amino acid structure as not all the members of the genus of proteins comprising part (a) and (b) as claimed in claim 13 are monitor proteins.

2.2.2. Scope of enablement

Rejection of claims 1-3 is moot because the claims have been cancelled.

Claims 13 and 4-5 and 9 are rejected under 35 U.S.C. 112, first paragraph, because the specification, while being enabling for monitoring protein consisting of for two green fluorescence proteins form *Aequorea victoria* attached to both ends of the phosphorylation region having amino acid sequence of SEQ ID NO: 1, wherein said protein is produced by expression of pETIC-ART plasmid, does not reasonably provide enablement for any pair of fluorescent proteins whose fluorescence is changed by phosphorylation of any phosphorylation region of any sequence of amino acids; see the above rejection for lack of written description. The scope of the claims covers any fusion protein, and method of its use, wherein said fusion protein comprises any phosphorylation region, or phosphorylation region of SEQ ID NO: 1, and any pair of fluorescent proteins or two green fluorescence proteins form *Aequorea victoria*, wherein

said pair of fluorescent protein shows a change of fluorescence caused by phosphorylation of the phosphorylation region.

The claims are broader in scope than the enablement set forth in the specification. The scope of the claims must bear a reasonable correlation with the scope of enablement (In re Fisher, 166 USPQ 19 24 (CCPA 1970)). Otherwise, undue experimentation is necessary to make the claimed invention. Factors to be considered in determining whether undue experimentation is required, are summarized *In re* Wands [858 F.2d 731, 8 USPQ 2nd 1400 (Fed. Cir. 1988)]. The Wands factors are: (a) the nature of the invention, (b) the breadth of the claim, (c) the state of the prior art, (d) the relative skill of those in the art, (e) the predictability of the art, (f) the presence or absence of working example, (g) the amount of direction or guidance presented, (h) the quantity of experimentation necessary.

The nature and breath of the claimed invention encompasses any fusion protein that shows change of fluorescence of fluorescent proteins caused by phosphorylation of its phosphorylation region. The components of the fusion protein have to be from any organism or man-made. Although production of hybrid protein is well known in the art and skills of artisan high, to make an use the claimed invention is not in the realm of routine experimentation. Applicants provide only the guidance regarding the use of two green proteins from *Aequorea victoria* and SEQ ID NO: 1 as components of the fusion monitor protein. Applicants themselves provided evidence that some fusion proteins such as Kempart, do not work as monitor protein; see the above rejection for lack of written description. Thus, it is unpredictable what phosphorylation region and

fluorescence proteins should be fused such that there will be a change in the fluorescence of fusion protein after phosphorylation of the phosphorylation region. The disclosure does not teach how to select phosphorylation region and fluorescent proteins. Thus, one skilled in the art is forced to make a DNA construct encoding a phosphorylated region flanked by fluorescent proteins, express said construct and test the protein for the property of being phosphorylated as well as being able to change the fluorescence in response to phosphorylation. Without further guidance on the part of Applicants as to the nature and structure of the phosphorylation region and fluorescent proteins and the means of their connection, i.e. full structural description the fusion protein that is the monitoring protein as well, experimentation left to those in the art is improperly extensive and undue.

In traversing the rejection for the scope of enablement Applicants write on page 20, line 8,

> "It would not require undue experimentation to test if any of these sequences can be used in a monitor protein. The specification teaches how to test if an amino acid sequence can be used as a phosphorylation region,"

and further, in line 19, Applicants state,

> "The specification also provides detailed instruction for performing fluorescence measurements in Example 5."

Applicants arguments have been fully considered but are found not persuasive, because the experimentation, as indicated in the above rejection for the scope of

enablement, is not limited to testing whether the region chosen as the phosphorylation region is phosphorylated and by which kinase, and performing fluorescence measurements of the fusion protein. The undue experimentation comprises making a DNA construct encoding a phosphorylated region flanked by fluorescent proteins, expressing said construct and testing the fusion protein for the property of being phosphorylated as well as being able to change the fluorescence in response to phosphorylation.

3.3. 35 USC section 102

The rejection of claims 1 and 2, under 35 U.S.C. 102(b) as being anticipated by the US Patent No. 5,925,558 is moot because the claims have been canceled.

The rejection of claim 9 made in the previous Office Action is withdrawn, because the claim has been amended.

3.4. 35 USC section 103

The rejection of claims 4 and 5 made in the previous Office Action is withdrawn, because the claims have been amended.

4. Conclusion

No claim is in condition for allowance, however, the Application contains allowable subject matter. The following is the examiner's reasons for indicating allowable subject matter.

Applicants are the first to disclose a fusion protein for monitoring of phosphorylase activity in a sample by measurements of change resonance energy transfer of two fluorescent proteins. The fusion protein consist of SEQ ID NO:1 and two green proteins from *Aequorea victoria*. The invention is free of prior art and nonobvious, because one cannot predict which fusion protein containing two green proteins from *Aequorea victoria* and a phosphorylation region will act as a monitoring protein. The closest prior art is the US Patent 5,925,558 which describes a monitoring fusion protein consisting of phosphorylation region and one *Aquefora* related fluorescent protein. The phosphorylation regions used in the Patent are different than that of SEQ ID NO: 1 of the instant application.

THIS ACTION IS MADE FINAL. Applicant is reminded of the extension of time policy as set forth in 37 CFR 1.136(a).

A shortened statutory period for reply to this final action is set to expire THREE MONTHS from the mailing date of this action. In the event a first reply is filed within TWO MONTHS of the mailing date of this final action and the advisory action is not mailed until after the end of the THREE-MONTH shortened statutory period, then the shortened statutory period will expire on the date the advisory action is mailed, and any extension fee pursuant to 37 CFR 1.136(a) will be calculated from the mailing date of the advisory action. In no event, however, will the statutory period for reply expire later than SIX MONTHS from the mailing date of this final action.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Malgorzata A. Walicka, Ph.D., whose telephone number

is (571) 272-0944 and the right fax number is (571) 273-0944. The examiner can normally be reached Monday-Friday from 10:00 a.m. to 4:30 p.m. EST.

If attempts to reach examiner by telephone are unsuccessful, the examiner's supervisor, Ponnathapura Achutamurthy, Ph.D. can be reached on (571) 272-0928. The fax phone number for this Group is 703.872.9306.

Any inquiry of a general nature or relating to the status of this application should be directed to the Group receptionists whose telephone number is (703) 308-0196.

Malgorzata A. Walicka, Ph.D.

Art Unit 1652

Patent Examiner

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